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Declaration under Rule 4.17:

— of inventorship (Rule 4.17(iv)) for US only

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(54) Title: SELF-PROCESSING PLANTS AND PLANT PARTS

(57) Abstract: The invention provides polynucleotides, preferably synthetic polynucleotides, which encode processing enzymes that are optimized for expression in plants. The polynucleotides encode mesophilic, thermophilic, or hyperthermophilic processing enzymes, which are activated under suitable activating conditions to act upon the desired substrate. Also provided are "self-processing" transgenic plants, and plant parts, e.g., grain, which express one or more of these enzymes and have an altered composition that facilitates plant and grain processing. Methods for making and using these plants, e.g., to produce food products having improved taste and to produce fermentable substrates for the production of ethanol and fermented beverages are also provided.

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## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12N 15/31,15/56,15/62,15/82,15/84; C12P 7/06,19/00; A01H 5/00,5/10  
 US CL : 800/278,284,287,288,294,317.4,320.1; 435/69.8,101,161,202,252.2,320.1,412,419,468,469; 536/23.2,23.7

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 800/278,284,287,288,294,317.4,320.1; 435/69.8,101,161,202,252.2,320.1,412,419,468,469; 536/23.2,23.7

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ROGERS et al. Isolation and Sequence Analysis of a Barley Alpha-Amylase cDNA Clone. The Journal of Biological Chemistry. July 1983, Vol. 258, No. 13, pages 8169-8174, especially page 8173, Figure 5.	1-4,16,35,41,43 -----
--		5,17-29,42,44-50,60- 61,73-75,82-86,92- 95,97-98,100-101,103- 104,108-109,122- 131,133-134,156- 159,169,178-187,195- 196,214-217,233
Y		

Further documents are listed in the continuation of Box C.

See patent family annex.

*	Special categories of cited documents:	
"A"	document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"B"	earlier application or patent published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

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**C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 92/05259 A1 (GIST-BROCADES, N.V.) 02 April 1992 (02.04.2002), pages 21-23 and 27-28.	1-2,16- 17,19,23,28,35,41- 45,47,49-50,60-61,73- 74,82,92,95,97,156- 157,169,214-215,217
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Y		5,18,20-22,24- 27,29,46-48,75,83- 86,93-94,98,100- 101,103-104,108- 109,122-131,133- 134,158-159,216 and 233
Y	5,366,883 A (ASADA et al) 22 November 1994 (22.11.1994), column 1; column 3, lines 36-46; column 9, lines 15-55; claims 1-6,8,11-12.	52,75,83,85,109,127- 128,130- 131,134,158,182,184, 186,216,233
Y	WO 98/39461 A1 (HOWARD, John A.) 11 September 1998 (11.09.1998), page 7, line 14 through page 8, line 31; page 10, lines 7-23; page 11, lines 3-15.	3-5,19-20,24- 27,46,60-61,84-86,94- 95,100-101,103- 104,108-109,122- 131,133-134,159,233
Y	WO 9732986 A2 (FRIEDRICH WEISSHEIMER MALZFABRIK) 12 September 1997 (12.09.1997), page 2, bottom paragraph through page 6, first full paragraph; page 10, bottom two paragraphs; page 11, top paragraph; page 12, bottom paragraph through page 14, second full paragraph; page 15, bottom paragraph through page 16, top paragraph; page 19, bottom paragraph through page 21; page 24.	3-5,19-21,24- 29,46,48,60-61,84- 86,93-5,98,100- 101,103-104,108- 109,122-131,133- 134,156-159,169,178- 187,195-196,214- 217,233
Y	US 6,013,860 A (HIMMEL et al) 11 January 2000 (11.01.2000), column 1, lines 14-35 and 49-55; column 2, lines 53-58.	178-187,195-196

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**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3.  Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**This International Searching Authority found multiple inventions in this international application, as follows:  
Please See Continuation Sheet

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.: Please See Continuation Sheet
  
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

  

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

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**BOX III. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING**

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-6,11,16-30,35-36,41-52,60-62,73-75,82-87,92-95,97-105,108-109,122-136,156-159,164,169-170,214-217 and 233-234, drawn to an isolated polynucleotide encoding alpha-amylase, a method of its use to transform plants, and plants transformed therewith.

Group II, claim(s) 1-5,7,12,16-29,34-35,37,41-50,53,60-61,63,73-75,82-86,88,92-95,97-105,108-109,122-127,129-131,156-159,168-170, and 214-217, drawn to an isolated polynucleotide encoding pullulanase, methods of its use to transform plants, and plants transformed therewith.

Group III, claim(s) 1-5,8,13-16-29,31,35,38,41-50,54-55,60-61,64,73-75,82-86,89,92-95,97-105,108-109,122-127,129-131,156-159,165,169-170 and 214-217, drawn to an isolated polynucleotide encoding alpha-glucosidase, a method of its use to transform plants, and plants transformed therewith.

Group IV, claim(s) 1-5,9,14,16-29,32,35,39,41-50,56-57,60-61,65,73-75,82-86,90,92-95,97-105,108-109,122-127,129-131,156-159,166,169-170 and 214-217, drawn to an isolated polynucleotide encoding glucose isomerase, a method for its use to transform plants, and plants transformed therewith.

Group V, claim(s) 1-5,10,15-29,33,35,40-50,58-61,66,73-75,82-86,91-95,97-105,108-109,122-127,129-131,156-159,167,169-170 and 214-217, drawn to an isolated polynucleotide encoding glucoamylase, a method for its use to transform plants, and plants transformed therewith.

Group VI, claim(s) 67-72,76-81 and 160-163, drawn to a plant transformed with a gene encoding a non-starch degrading enzyme.

Group VII, claim(s) 96, drawn to an isolated starch processing enzyme.

Group VIII, claim(s) 106-107, drawn to a composition comprising isolated starch and an enzyme.

Group IX, claim(s) 110-117, drawn to a method for preparing starch granules comprising treating isolated starch granules comprising an enzyme under conditions that activate the enzyme.

Group X, claim(s) 118-119, drawn to isolated starch produced by activating starch processing enzymes present in isolated starch granules.

Group XI, claim(s) 120-121, drawn to sugars produced by chemical treatment of starch produced from isolated starch granules.

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Group XII, claim(s) 137-155,218-220 and 229-232, drawn to methods for hydrolyzing starch produced by treating isolated starch granules from plants transformed with more than one gene encoding more than one processing enzyme, further comprising treating a plant part with a topically applied enzyme.

Group XIII, claim(s) 171-172, drawn to isolated starch produced from a plant transformed with a starch processing enzyme gene.

Group XIV, claim(s) 173-174, drawn to a method for hydrolyzing a plant transformed with a non-starch processing enzyme gene for the production of a solution of sugars.

Group XV, claim(s) 175-177, drawn to a method of treating transformed seeds with a protease or lipase enzyme to release an aqueous mixture which comprises a processing enzyme encoded by a transgene in the transformed plant.

Group XVI, claim(s) 178-188 and 195-196, drawn to a method for preparing ethanol from a plant transformed with an alpha-amylase gene.

Group XVII, claim(s) 178-187, 189 and 195-196, drawn to a method for preparing ethanol from a plant transformed with an alpha-glucosidase gene.

Group XVIII, claim(s) 178-187, 190 and 195-196, drawn to a method for preparing ethanol from a plant transformed with a glucose isomerase gene.

Group XIX, claim(s) 178-187, 191 and 195-196, drawn to a method for preparing ethanol from a plant transformed with a glucoamylase gene.

Group XX, claim(s) 178-187, 192 and 195-196, drawn to a method for preparing ethanol from a plant transformed with a pullulanase gene.

Group XXI, claim(s) 193-194, drawn to a method for producing ethanol from a plant transformed with a non-starch processing enzyme gene.

Group XXII, claim(s) 197-207, drawn to a method for producing a sweetened farinaceous food product from a transformed plant comprising a gene encoding a starch processing enzyme.

Group XXIII, claim(s) 208-211, drawn to a method for sweetening a starch-containing non-food product such as glue produced from a plant transformed with a gene encoding a starch processing enzyme.

Group XXIV, claim(s) 212, drawn to a farinaceous food product.

Group XXV, claim(s) 213, drawn to a starch-containing non-food product.

Group XXVI, claim(s) 221-222, drawn to a method for isolating an enzyme from a cultured transformed plant cell or plant.

Group XXVII, claim(s) 223-226, drawn to a process for making maltodextrin.

Group XXVIII, claim(s) 227-228, drawn to isolated maltodextrin.

Furthermore, within the elected groups above, a further lack of unity exists between each claim drawn to each isolated polynucleotide encoding a different amino acid sequence. Upon electing a group above, Applicant is required to select a single polynucleotide from the polynucleotides encoding the following 44 amino acid sequences, corresponding to Groups XXIX-LXXII, respectively: SEQ ID NO: 1,3,5,8,10,13-16,18,20,24,26-30,33-36,38,40,42,44,45,47,49,51,62,64,66,70,80,82,84,86,88,90,92,100,108,110 or 112.

The inventions listed as Groups I-LXXII do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The claims are linked by the technical feature of a polynucleotide encoding a starch processing enzyme such as alpha-amylase or glucoamylase, methods for plant transformation therewith, and the resultant transformed plants or plant parts which produce altered starch. However, this feature is not special because it does not constitute an advance over the prior art. WO 92/05259 A1 (GIST-BROCADES) published 02 April 1992 teaches transformed potato plants containing glucoamylase and alpha-amylase genes, wherein said plants produce altered starch (see, e.g., pages 27-28).

Furthermore, each Group is drawn to a physiologically and biochemically divergent product, such as an isolated polynucleotide of a particular sequence encoding an enzyme of particular activity, transformed plants, isolated starches, isolated proteins, isolated sugars, Form PCT/ISA/210 (extra sheet) (January 2004)

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food products, non-food starch-containing products, etc., each not required by the other. In addition, each Group is drawn to a different process involving different starting materials, reagents, process steps, and final products, each not required by the other. Finally, the Groups encompass multiple products and multiple methods of using them and making them.

Continuation of Box III Item 3:

1-5,16-29,35,41-50,60-61,73-75,82-86,92-95,97-98,100-101,103-104,108-109,122-131,13-134,156-159,169,178-187,195-196,214-217 and 233, corresponding to Groups I and XVI, and SEQ ID NOS:87-88.